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(71) Applicant *(for all designated States except US)*: COMPUGEN LTD. [IL/IL]; Pinchas Rozen Street 72, 69512 Tel-Aviv (IL).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: MINTZ, Liat [IL/IL]; Harav Kook Street 14, 47283 Ramat Hasharon (IL). SAVITSKY, Kinneret [IL/IL]; Jacob Street 16, Tel Baruch, 69015 Tel-Aviv (IL).

(74) Agent: REINHOLD COHN AND PARTNERS; P.O. Box 4060, 61040 Tel-Aviv (IL).

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(54) Title: ANGIOPOIETIN-LIKE GROWTH FACTOR SEQUENCES

(57) Abstract

The present invention concerns novel nucleic acid and amino acid sequences, expression vectors, host cells and pharmaceutical compositions comprising same, as well as antibodies directed against said amino acid sequences and methods for detecting the amino acid sequences and the nucleic acid sequences in a sample.

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ANGIOPOIETIN-LIKE GROWTH FACTOR SEQUENCES

FIELD OF THE INVENTION

This invention relates to novel polypeptide and polynucleotide compositions, to the production of these compositions, and to the use of the compositions in the diagnosis, prevention, and treatment of disease states.

BACKGROUND OF THE INVENTION

Growth factors are a large and diverse group of polypeptides that coordinate numerous cellular interactions. They are secreted proteins, varying in molecular weight from about 5 kDal to over 50 kDal, that act on nearby cells in a paracrine or autocrine manner. Some growth factors also behave like hormones and are transported through the blood stream to distant target organs.

Growth factors have varied effects, acting not only as regulators of cell proliferation but also as secretogogues, chemoattractants and differentiation factors, and play a role in tissue remodeling. Tissue remodeling occurs during embryonic development, somatic growth, reproductive maturation, and in response to injury, inflammation, and neoplastic transformation. All these processes involve cell proliferation (hyperplasia) and extension, enlargement of existing cells (hypertrophy), and apoptosis or programmed cell death. A major role of growth factors is to coordinate the activities of the different cell types in the remodeled tissues.

Growth factors tend to be either highly localized in the responsive tissue(s), or are present at very low concentrations in plasma, so that purification and identification of new growth factors is sometimes difficult. Growth factors are very powerful regulatory agents, active at sub-nanomolar concentrations. Thus, many growth factors are currently utilized as potent therapeutic agents, and

intensive efforts are underway to identify new growth factors.

Growth factors act by binding to cells through transmembrane receptors. Growth factor receptors include the protein tyrosine kinases (PTKs), which transduce signals which regulate cell growth and differentiation by the phosphorylation of various intracellular proteins. Examples of PTKs include fibroblast growth factor receptor and nerve growth factor receptor.

One family of growth factor proteins is characterized by a C-terminal region homologous to the C-terminal domain of fibrinogen. The *"fibrinogen-like domains"* are suggested to be involved in protein-protein interactions (Xu X. and Doolittle R.F., *Proc. Natl. Acad. Sci.* U.S.A. **87**:2097-2101, (1990)). These domains are found in the C-termini of several proteins involved in tissue development, including: scabrous (Sca), a secreted glycoprotein which is involved in the regulation of neurogenesis in Drosophila (Lee, E.C. *et al. Mol. Cell Biol.* **16**:1179-1188, (1996)); the tenascins TN (cytotactin), TN-R, and TN-C, extracellular matrix proteins which are involved in embryogenesis and development of mammalian epithelia, cartilage, bone, and neuronal tissue (Erickson H.P. *et al., Annu. Rev. Cell. Biol.* **5**:71-92, (1989)) ; ficolin-α and -β, which bind transforming growth factor-β1 (TGF-β1; Ichijo H. *et al., J. Biol. Chem.* **19**:14505-14513, (1993)); and angiopoietin-1 and -2, which are involved in vascular morphogenesis and maintenance, vascular remodeling, and angiogenesis (Davis S. *et al., Cell*, **87**:1161-1169, (1996); Suri C. *et al., Cell* **87**:1171-1180, (1996); Maisonpierre, P.C. *et al., Science*, **277**:55-60, (1997)).

The TIE-1 and TIE-2 receptor tyrosine kinases are expressed on endothelial and early hemopoietic cells (Dumont, D.J., *et al., Oncogene,* **7**:1471-1480, (1992); Schnurch, H. and Risau, W., *Development* **119**:957-968, (1993)). TIE-2 appears to mediate vessel remodeling and maturation, and is involved in both angiogenesis and in vascular maintenance (Wong, A.L., *et al., Circ. Res.*, **81**:567-574, (1997)). The TIE-1 signal is implicated in the control of fluid exchange across capillaries and in hemodynamic stress resistance (Sato, *et al., Nature,* **376**:70, (1995); Puri, *et al., EMBO J.* **14**:5884, (1995)). The

physiological ligands of the TIE-1 receptor tyrosine kinase have yet to be described.

Angiopoietin-1 (Ang-1; Davis, S. *et al., supra*; Suri C. *et al., supra*) and its natural antagonist angiopoietin-2 (Ang-2; Maisonpierre, P.C. *et al., supra*) have recently been identified as the physiological ligands of TIE-2. Ang-1 and Ang-2 contain 498 and 496 amino acids, respectively, and share approximately ~60% sequence identity. Both are secreted glycoproteins with an N-terminal coiled-coil domain and a C-terminal fibrinogen-like domain. Ang-1 expression is highest in the heart in the early stages of development, and is widely expressed in adult tissues. In contrast, Ang-2 is expressed only at sites of vascular remodeling in the normal adult (such as in ovary, placenta and uterus). While Ang-1 binding to TIE-2 solidifies and stabilizes newly formed blood vessels, the Ang-2 negative signal causes vessel structures to become loosened, reducing endothelial cell contacts with matrix and disassociating peri-endothelial support cells.

The importance of angiogenesis in the growth of solid tumors and metastasis is well recognized. In the absence of access to an adequate vasculature tumor growth is restricted to a diameter of approximately 0.4 mm, and tumor cells become necrotic and/or apoptotic (Gimbrone *et al., J. Exp. Med.*, 136:261-276, (1972); Hanahan, D. and Folkman, J., *Cell*, 86:353-364, (1996)). Without neovascularization the metastatic tumor cells may form microscopic dormant colonies with equally high replication and death rates (Folkman, J. *Nature Med.*, 1:27-31, (1995)). Angiogenesis inhibitors are therefore very promising therapeutics for neoplastic diseases as well as other diseases involving neovascularization control.

New growth factors having similarity to angiopoietins and other fibrinogen-like growth factors have the potential to modulate tissue growth, development, and remodeling processes, such as vasculogenesis, angiogenesis, neurogenesis, and hemopoiesis. Such growth factors can contribute to the therapeutic manipulation of, for example, vessel growth, either positively, to promote wound healing, tissue regeneration, and revascularization in diseases

characterized by vascular insufficiency, or negatively, to prevent tumor growth and other disease processes driven by angiogenesis, such as atherosclerosis, diabetic retinopathy, and arthritis.

Therefore, discovery of new growth factor proteins having similarity to angiopoietins and other fibrinogen-like growth factors, and the polynucleotides which encode them, satisfies a need in the art by providing new compositions which are useful in treatment of various conditions, such as wound healing and tissue regeneration, and the diagnosis, prevention, and treatment of various disease states, including malignant, proliferative, inflammatory, and immune conditions and disorders.

SUMMARY OF THE INVENTION

The invention includes novel, isolated angiopoietin-like growth factors, termed hereinafter collectively as *"ALGF"*. The sequences are derived from two genes: one from muscle (muscle ALGF), and one from liver (liver ALGF), and may be of human or mouse origin. ALGF contains an amino acid sequence having at least an 80 percent identity with any one of the sequences depicted with SEQ ID NOS:5 to 8. In other embodiments, ALGF comprises a sequence at least 90% identical to SEQ ID NO:5 to 8, or comprises a sequence substantially identical to SEQ ID NO:5 to 8.

The invention also includes (i) fragments of ALGF, preferably at least about 20 amino acids in length, more preferably at least about 50 amino acids in length; and (ii) a pharmaceutical composition containing ALGF. In various embodiments, the fragment corresponds to the N-terminal domain of ALGF or the C-terminal fibrinogen-like domain of all the ALGFs' sequences.

In another aspect the invention includes an isolated polynucleotide having a sequence which encodes ALGF as described above, or a sequence complementary to the ALGF coding sequence, and a composition comprising the polynucleotide. The polynucleotide may be mRNA, cRNA, DNA, cDNA, genomic DNA, as well as an antisense analog thereof. The polynucleotide may

encode a ALGF having at least 80% sequence identity to any one of the amino acid sequence of SEQ ID NO:5 to 8. The polynucleotide may contain, for example, a coding sequence having at least 80% sequence identity with the polynucleotide sequence identified as any one of SEQ ID NO:1 to SEQ ID NO:4. In a specific embodiment, the polynucleotide comprises a sequence substantially identical to any one of SEQ ID NO:1 to SEQ ID NO:4. The composition also contemplates fragments of the polynucleotide, preferably at least about 15 nucleotides in length, preferably at least 20 or 30 nucleotides in length.

Also disclosed is a recombinant expression vector containing a polynucleotide encoding ALGF or a fragment described above, and, operably linked to the polynucleotide, regulatory elements effective for expression of the protein in a selected host. Preferred coding sequences are given above. In a related aspect, the invention includes a host cell containing the vector.

The invention further includes a method for producing ALGF by recombinant techniques, by culturing recombinant prokaryotic or eukaryotic host cells containing nucleic acid sequence encoding ALGF under conditions promoting expression of the polypeptide, and subsequent recovery of the polypeptide from the host cell or the cell culture medium.

In another aspect, the present invention concerns methods for treating diseases or disorders which show a beneficial effect (i.e. can be prevented, ameliorated or cured) by increasing the level of the ALGF in the body of the individual in need of such treatment.

According to said method, the invention provides methods for promoting wound healing comprising administering to a subject an effective amount of a pharmaceutical composition comprising purified ALGF.

Another example in accordance with said method concerns a method for inhibiting tumor growth comprising administering to a subject an effective amount of a pharmaceutical composition comprising purified ALGF.

In still another aspect, the invention includes an antibody specific against ALGF. The antibody has diagnostic and therapeutic applications, particularly in

treating conditions where a beneficial effect can be achieved by decreasing the level of ALGF in the patient such as in treating tumors, other proliferative and malignant disorders, and immune and inflammatory disorders. Treatment methods which employ antisense or coding sequence polynucleotides for inhibiting or enhancing levels of ALGF are also contemplated, as are treatment methods which employ antibodies specific against ALGF.

Diagnostic methods for detecting levels of ALGF in specific tissue samples, and for detecting levels of expression of ALGF in tissues, also form part of the invention. In one embodiment, a method of detecting a polynucleotide which encodes ALGF in a biological sample, involves the steps of: (a) hybridizing the complement of a polynucleotide which encodes ALGF to nucleic acid material of a biological sample, thereby forming a hybridization complex, and (b) detecting the hybridization complex, wherein the presence of the complex correlates with the presence of a polynucleotide encoding ALGF in the biological sample. Methods for detecting splice variants of ALGF and mutations in the coding region of ALGF are also contemplated.

Screening methods which employ ALGF for identifying a receptor which binds ALGF also form part of the invention. An exemplary method includes (a) contacting ALGF with a cell expressing a cell-surface receptor molecule, (b) measuring the cellular response in the presence of ALGF, (c) selecting the receptor expressed by the cell as a candidate ALGF receptor if the cellular response effected by the receptor in the presence of ALGF is above a selected threshold level. The cellular response measured may be, for example, tyrosine phosphorylation, cellular proliferation, differentiation, or cell migration.

The invention also includes, in a related aspect, a receptor identified by the screening methods described above.

Screening methods which employ ALGF for identifying a compound which modulates the activity of the ALGF receptor also form part of the invention. An exemplary method includes (a) contacting ALGF and a test compound with a cell expressing a cell-surface receptor responsive to ALGF, (b)

measuring the modulation of the cellular response by the test compound, and (c) selecting the test compound as candidate compound if the modulation of the cellular response is above a selected threshold level. The cellular response measured may be, for example, tyrosine phosphorylation, cellular proliferation, differentiation, or cell migration.

These and other objects and features of the invention will become more fully apparent when the following detailed description is read in conjunction with the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

In order to understand the invention and to see how it may be carried out in practice, a preferred embodiment will now be described, by way of non-limiting example only, with reference to the accompanying drawings, in which:

Fig. 1 shows an alignment between SEQ. ID NOS. 1 to 4 of the inventions and other similar genes.

BRIEF DESCRIPTION OF THE SEQUENCES

SEQ ID NO:1 shows the nucleic acid sequence of the muscle ALGF from human origin;

SEQ ID NO:2 shows the nucleic acid sequence of muscle ALGF from mouse origin;

SEQ ID NO:3 shows the nucleic acid sequence of liver ALGF from human origin;

SEQ ID NO:4 shows the nucleic acid sequence of liver ALGF from mouse origin;

SEQ ID NO:5 shows the predicted amino acid translation of SEQ ID NO:1;

SEQ ID NO:6 shows the predicted amino acid translation of SEQ ID NO:2;

SEQ ID NO:7 shows the predicted amino acid translation of SEQ ID NO:3;

SEQ ID NO:7 shows the predicted amino acid translation of SEQ ID NO:4;

DETAILED DESCRIPTION OF THE INVENTION

I. Definitions

Unless otherwise indicated, all terms used herein have the same meaning as they would to one skilled in the art of the present invention. Practitioners are particularly directed to Sambrook *et al.* (1989) *Molecular Cloning*: A Laboratory Manual (Second Edition), Cold Spring Harbor Press, Plainview, N.Y. and Ausubel FM *et al.* (1989) *Current Protocols in Molecular Biology*, John Wiley & Sons, New York, N.Y., for definitions and terms of the art. It is to be understood that this invention is not limited to the particular methodology, protocols, and reagents described, as these may vary.

An ***"angiopoietin-like growth factor"*** or ***"ANG-like growth factor"*** is a polypeptide which contains the fibrinogen beta and gamma chains C-terminal domain signature pattern FIBRIN_AG_C_DOMAIN and has at least 30% amino acid sequence identity to a corresponding aligned region of a mammalian angiopoietin-1 or angiopoietin-2.

"ALGF" refers collectively to ANG-like growth factors which can be muscle originating or a liver originating from human or mouse sources, containing a polypeptide having at least 80 percent, preferably at least 90 percent, and more preferably at least 95 percent sequence identity to any one of the sequences depicted in SEQ ID NO:5 to SEQ ID NO:8. The polypeptide may be a mature ALGF protein and/or a modified ALGF protein. As used herein, reference to ALGF is meant to include the full-length molecule as well as fragments thereof unless the context indicates otherwise.

The term ***"mature ALGF protein"*** refers to the ALGF protein as it exists in the cell after post-translational processing, *e.g.* removal of a signal sequence.

The term ***"modified"***, when referring to a protein of the invention, means a protein which is modified either by natural processes, such as processing or other post-translational modifications, or by chemical modification techniques which are well known in the art. Among the numerous known modifications which may be present include, but are not limited to, acetylation, acylation, amidation, ADP-ribosylation, glycosylation, GPI anchor formation, covalent attachment of a lipid or lipid derivative, methylation, myristlyation, pegylation, prenylation, phosphorylation, ubiqutination, or any similar process.

The term ***"biologically active"*** refers to a ALGF having structural, regulatory or biochemical functions of the naturally occurring ALGF such as the ability to bind to a receptor, preferably a PTK receptor, the ability to modulate intracellular protein phosphorylation, or the ability to modulate the generation of intracellular signals. Likewise, ***"immunologically active"*** defines the capability of a natural, recombinant or synthetic ALGF or any fragment thereof, to induce a specific immune response in appropriate animals or cells and to bind with specific antibodies.

The term ***"fragment"***, when referring to ALGF, means a polypeptide which has an amino acid sequence which is the same as part of but not all of the amino acid sequence of a ALGF protein, which either retains essentially the same biological function or activity as ALGF, or retains at least one of the functions or activities of ALGF; for example, a fragment which retains the ability to bind to a receptor, or a fragment which retains immunological activity of ALGF. The fragment preferably includes at least 20 contiguous amino acid residues of ALGF, more preferably at least 50 residues.

The term ***"portion"***, when referring to a protein of the invention, means a polypeptide which has an amino acid sequence which is the same as part of the amino acid sequence of the present invention or a variant thereof, which does not necessarily retain any biological function or activity.

A ***"conservative substitution"*** refers to the substitution of an amino acid in one class by an amino acid in the same class, where a class is defined by

common physicochemical amino acid sidechain properties and high substitution frequencies in homologous proteins found in nature (as determined, e.g., by a standard Dayhoff frequency exchange matrix or BLOSUM matrix). Six general classes of amino acid sidechains, categorized as described above, include: Class I (Cys); Class II (Ser, Thr, Pro, Ala, Gly); Class III (Asn, Asp, Gln, Glu); Class IV (His, Arg, Lys); Class V (Ile, Leu, Val, Met); and Class VI (Phe, Tyr, Trp). For example, substitution of an Asp for another class III residue such as Asn, Gln, or Glu, is a conservative substitution.

A ***"non-conservative substitution"*** refers to the substitution of an amino acid in one class with an amino acid from another class; for example, substitution of an Ala, a class II residue, with a class III residue such as Asp, Asn, Glu, or Gln.

"Optimal alignment" is defined as an alignment giving the highest percent identity score. Such alignment can be performed using a variety of commercially available sequence analysis programs, such as the local alignment program LALIGN using a ktup of 1, default parameters and the default PAM. A preferred alignment is the one performed using the CLUSTAL-W program in MacVector, operated with default parameters, including an open gap penalty of 10.0, an extended gap penalty of 0.1, and a BLOSUM similarity matrix. If a gap needs to be inserted into a first sequence to optimally align it with a second sequence, the percent identity is calculated using only the residues that are paired with a corresponding amino acid residue (i.e., the calculation does not consider residues in the second sequences that are in the *"gap"* of the first sequence).

"Percent sequence identity", with respect to two amino acid or polynucleotide sequences, refers to the percentage of residues that are identical in the two sequences when the sequences are optimally aligned. Thus, 80% amino acid sequence identity means that 80% of the amino acids in two optimally aligned polypeptide sequences are identical.

A first polypeptide region is said to *"correspond"* to a second polypeptide region when the regions are essentially co-extensive when the sequences

containing the regions are aligned using a sequence alignment program, as above. Corresponding polypeptide regions typically contain a similar, if not identical, number of residues. It will be understood, however, that corresponding regions may contain insertions or deletions of residues with respect to one another, as well as some differences in their sequences.

The term ***"sequence identity"*** means nucleic acid or amino acid sequence identity in two or more aligned sequences, aligned as defined above.

"Sequence similarity" between two polypeptides is determined by comparing the amino acid sequence and its conserved amino acid substitutes of one polypeptide to the sequence of a second polypeptide. Thus, 80% protein sequence similarity means that 80% of the amino acid residues in two or more aligned protein sequences are conserved amino acid residues, i.e. are conservative substitutions.

The term ***"gene"*** as used herein means the segment of DNA involved in producing a polypeptide chain; it may include regions preceding and following the coding region, e.g. 5' untranslated (5' UTR) or ***"leader"*** sequences and 3' UTR or "trailer" sequences, as well as intervening sequences (introns) between individual coding segments (exons).

An ***"isolated polynucleotide having a sequence which encodes ALGF"*** is a polynucleotide which contains the coding sequence of ALGF (i) in isolation, (ii) in combination with additional coding sequences, such as fusion protein or signal peptide, in which the ALGF coding sequence is the dominant coding sequence, (iii) in combination with non-coding sequences, such as introns and control elements, such as promoter and terminator elements or 5' and/or 3' untranslated regions, effective for expression of the coding sequence in a suitable host, and/or (iv) in a vector or host environment in which the ALGF coding sequence is a heterologous gene.

The terms ***"heterologous DNA"*** and ***"heterologous RNA"*** refer to nucleotides that are not endogenous to the cell or part of the genome in which they are present; generally such nucleotides have been added to the cell. by

transfection, microinjection, electroporation, or the like. Such nucleotides generally include at least one coding sequence, but this coding sequence need not be expressed.

The term ***"isolated"*** means that the material is removed from its original environment (e.g., the natural environment if it is naturally occurring). For example, a naturally-occurring polynucleotide or polypeptide present in a living animal is not isolated, but the same polynucleotide or polypeptide, separated from some or all of the coexisting materials in the natural system, is isolated. Such polynucleotides could be part of a vector and/or such polynucleotides or polypeptides could be part of a composition, and still be isolated in that such vector or composition is not part of its natural environment.

The term ***"fragment"***, when referring to a ALGF coding sequence, means a polynucleotide which has a nucleic acid sequence which is the same as part of but not all of the nucleic acid sequence of the ALGF coding sequence. The polynucleotide fragment preferably includes at least 15 contiguous nucleotides of ALGF coding sequence, preferably at least 20 or 30 nucleotides.

The term ***"expression vector"*** refers to vectors that have the ability to incorporate and express heterologous DNA fragments in a foreign cell. Many prokaryotic and eukaryotic expression vectors are commercially available. Selection of appropriate expression vectors is within the knowledge of those having skill in the art.

The term ***"substantially purified"*** refers to molecules, either polynucleotides or polypeptides, that are removed from their natural environment, isolated or separated, and are at least 60% free, preferably 75% free, and most preferably 90% free from other components with which they are naturally associated.

A ***"variant"*** polynucleotide sequence encodes a *"variant"* amino acid sequence which is altered by one or more amino acids from the reference polypeptide sequence. The variant polynucleotide sequence may encode a variant amino acid sequence which contains *"conservative"* substitutions, wherein the

substituted amino acid has structural or chemical properties similar to the amino acid which it replaces. In addition, or alternatively, the variant polynucleotide sequence may encode a variant amino acid sequence which contains *"non-conservative"* substitutions, wherein the substituted amino acid has dissimilar structural or chemical properties to the amino acid which it replaces. Variant polynucleotides may also encode variant amino acid sequences which contain amino acid insertions or deletions, or both.

An ***"allelic variant"*** is an alternate form of a polynucleotide sequence which may have a substitution, deletion or addition of one or more nucleotides, which does not substantially alter the function of the encoded polypeptide.

"Alternative splicing" is a process whereby multiple polypeptide isoforms are generated from a single gene, and involves the splicing together of nonconsecutive exons during the processing of some, but not all, transcripts of the gene. Thus a particular exon may be connected to any one of several alternative exons to form messenger RNAs. The alternatively-spliced mRNAs produce polypeptides *("splice variants")* in which some parts are common while other parts are different.

"Splice variants" of ALGF, when referred to in the context of an mRNA transcript, are mRNAs produced by alternative splicing of coding regions, i.e., exons, from the ALGF genes (both liver and muscle genes, originating from human or mouse.)

"Splice variants" of ALGF, when referred to in the context of the protein itself, are ALGF translation products which are encoded by alternatively-spliced ALGF mRNA transcripts.

"Splice segments" are mRNA segments which are spliced together either consecutively, encoding ALGF, or non-consecutively, encoding ALGF splice variants. These segments may contain 5' untranslated regions, protein coding regions, and 3' untranslated regions.

A ***"mutant"*** amino acid or polynucleotide sequence is a variant amino acid sequence, or a variant polynucleotide sequence which encodes a variant

amino acid sequence, which has significantly altered biological activity from that of the naturally occurring protein.

A ***"deletion"*** is defined as a change in either nucleotide or amino acid sequence in which one or more nucleotides or amino acid residues, respectively, are absent.

An ***"insertion"*** or ***"addition"*** is that change in a nucleotide or amino acid sequence which has resulted in the addition of one or more nucleotides or amino acid residues, respectively, as compared to the naturally occurring sequence.

A ***"substitution"*** results from the replacement of one or more nucleotides or amino acids by different nucleotides or amino acids, respectively.

The term ***"modulate"*** as used herein refers to the change in activity of the polypeptide of the invention. Modulation may relate to an increase or a decrease in biological activity, binding characteristics, or any other biological, functional, or immunological property of the molecule.

The term ***"agonist"*** as used herein, refers to a molecule which, when bound to the receptor of the present invention, modulates the activity of the receptor by inducing, increasing, or prolonging the duration of the biological activity mediated by the receptor. Agonists may themselves be polypeptides, nucleic acids, carbohydrates, lipids, or derivatives thereof, or any other molecules which bind to and modulate the activity of the receptor.

The term ***"antagonist"*** as used herein, refers to a molecule which, when bound to the receptor of the present invention, modulates the activity of the receptor by blocking, decreasing, or shortening the duration of the biological activity mediated by the receptor. Antagonists may themselves be polypeptides, nucleic acids, carbohydrates, lipids, or derivatives thereof, or any other molecules which bind to and modulate the activity of the receptor.

The term ***"humanized antibody"*** refers to antibody molecule in which one or more amino acids have been replaced in the non-antigen binding regions in order to more closely resemble a human antibody, while still retaining the original binding activity of the antibody.

"Treating a disease" refers to administering a therapeutic substance effective to reduce the symptoms of the disease and/or lessen the severity of the disease.

II. Polynucleotides Encoding ALGF

A. ALFG

Fig. 1 shows an alignment between the sequences depicted in SEQ ID NO: 1 to SEQ ID NO. 4 and sequences of other known genes.

mang-liver is the liver ALGF from mouse (SEQ ID NO: 4);

hang-liver is the liver ALGF from human (SEQ ID NO: 3).

mang–muscle is the muscle ALGF from mouse (SEQ ID NO: 2)

hang-muscle is the muscle ALFG from human (SEQ ID NO: 1).

The accession number in GeneBank of the known genes are:

ang-1:AAB50557

ang-2: AAB63190

ANG3: AAD31728

ANG4:AAD31728

ctd6: CAA 76078

ang-y: AAD19608

Alignment between two sequences was carried out using the "pile-up" program version 10.0.

B. Polynucleotide compositions

The polynucleotides of the invention include sequences which encode ALGF, polynucleotide sequences complementary to the polypeptide coding sequence, and novel fragments of the polynucleotide. The polynucleotides may be in the form of RNA or in the form of DNA, and include mRNA, synthetic RNA and DNA, cRNA and cDNA, and genomic DNA. The polynucleotides may be double-stranded or single-stranded, and if single-stranded may be the coding strand or the non-coding (anti-sense, complementary) strand.

In a general embodiment, the polynucleotide has at least 70%, preferably 80% or 90% sequence identity with the at least one of the sequences identified as SEQ ID NOS:1 to 4. In another embodiment, the polynucleotide has a sequence essentially identical to any one of SEQ ID NOS:1 to 4.

The polynucleotides may include the coding sequence of ALGF (i) in isolation, (ii) in combination with additional coding sequences, such as those coding for a protein or signal peptide, in which the ALGF coding sequence is the dominant coding sequence, (iii) in combination with non-coding sequences, such as introns and control elements, such as promoter and terminator elements or 5' and/or 3' untranslated regions, effective for expression of the coding sequence in a suitable host, and/or (iv) in a vector or host environment in which the ALGF coding sequence is a heterologous gene.

The polynucleotides of the present invention may also have the protein coding sequence fused in-frame to a marker sequence which allows for purification of ALGF or its splice variants. The marker sequence may be, for example, a hexahistidine tag to provide for purification of the mature polypeptide fused to the marker in the case of a bacterial host, or, the marker sequence may be a hemagglutinin (HA) tag when a mammalian host, e.g. COS-7 cells, is used. The HA tag corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson, I., *et al., Cell* 37:767, (1984)).

Also contemplated are novel uses of polynucleotide fragments, also referred to herein as oligonucleotides, typically having at least 15 bases,

preferably at least 20 or 30 bases, corresponding to a region of the coding-sequence polynucleotide. The fragments may be used as probes, primers, antisense agents, and the like, according to known methods.

C. Preparation of polynucleotides

The polynucleotides may be obtained by screening cDNA libraries using oligonucleotide probes which can hybridize to or PCR-amplify polynucleotides which encode the ALGF protein and fragments disclosed above. cDNA libraries prepared from a variety of tissues are commercially available and procedures for screening and isolating cDNA clones are well-known to those of skill in the art. Such techniques are described in, for example, Sambrook *et al.* (1989) *Molecular Cloning:* A Laboratory Manual (2nd Edition), Cold Spring Harbor Press, Plainview, N.Y. and Ausubel FM *et al.* (1989) *Current Protocols in Molecular Biology*, John Wiley & Sons, New York, N.Y.

The polynucleotides may be extended to obtain upstream and downstream sequences such as promoters, regulatory elements, and 5' and 3' untranslated regions (UTRs). Extension of the available transcript sequence may be performed by numerous methods known to those of skill in the art, such as PCR or primer extension (Sambrook *et al., supra*), or by the RACE method using, for example, the Marathon RACE kit (Clontech, Cat. # K1802-1).

Alternatively, the technique of *"restriction-site"* PCR (Gobinda *et al., PCR Methods Applic.* 2:318-22, (1993)), which uses universal primers to retrieve flanking sequence adjacent a known locus, may be employed. First, genomic DNA is amplified in the presence of primer to a linker sequence and a primer specific to the known region. The amplified sequences are subjected to a second round of PCR with the same linker primer and another specific primer internal to the first one. Products of each round of PCR are transcribed with an appropriate RNA polymerase and sequenced using reverse transcriptase.

Inverse PCR can be used to amplify or extend sequences using divergent primers based on a known region (Triglia T *et al., Nucleic Acids Res* 16:8186. (1988)). The primers may be designed using OLIGO(R) 4.06 Primer Analysis

Software (1992; National Biosciences Inc, Plymouth, Minn.), or another appropriate program, to be 22-30 nucleotides in length, to have a GC content of 50% or more, and to anneal to the target sequence at temperatures about 68-72°C. The method uses several restriction enzymes to generate a suitable fragment in the known region of a gene. The fragment is then circularized by intramolecular ligation and used as a PCR template.

Capture PCR (Lagerstrom M *et al., PCR Methods Applic,* 1:111-19, (1991)) is a method for PCR amplification of DNA fragments adjacent to a known sequence in human and yeast artificial chromosome DNA. Capture PCR also requires multiple restriction enzyme digestions and ligations to place an engineered double-stranded sequence into a flanking part of the DNA molecule before PCR.

Another method which may be used to retrieve flanking sequences is that of Parker, JD *et al., Nucleic Acids Res* 19:3055-60, (1991)). Additionally, one can use PCR, nested primers and PromoterFinder™ libraries to *"walk in"* genomic DNA (Clontech, Palo Alto, CA). This process avoids the need to screen libraries and is useful in finding intron/exon junctions. Preferred libraries for screening for full length cDNAs are ones that have been size-selected to include larger cDNAs. Also, random primed libraries are preferred in that they will contain more sequences which contain the 5' and upstream regions of genes. A randomly primed library may be particularly useful if an oligo d(T) library does not yield a full-length cDNA. Genomic libraries are useful for extension into the 5' nontranslated regulatory region.

The polynucleotides and oligonucleotides of the invention can also be prepared by solid-phase methods, according to known synthetic methods. Typically, fragments of up to about 100 bases are individually synthesized, then joined to form continuous sequences up to several hundred bases.

D. Applications of polynucleotides

The polynucleotide coding sequences and novel oligonucleotides of the invention have a variety of uses in (1) synthesis of ALGF, (2) diagnostics, (3) gene mapping, and (4) therapeutics.

D1. Synthesis of ALGF protein and fragments

In accordance with the present invention, polynucleotide sequences which encode ALGF, fragments of the protein, fusion proteins, or functional equivalents thereof, collectively referred to herein as *"ALGF"*, may be used in recombinant DNA molecules that direct the expression of ALGF in appropriate host cells. Due to the inherent degeneracy of the genetic code, other nucleic acid sequences which encode substantially the same or a functionally equivalent amino acid sequence may be used to clone and express ALGF.

As will be understood by those of skill in the art, it may be advantageous to produce ALGF-encoding nucleotide sequences possessing non-naturally occurring codons. Codons preferred by a particular prokaryotic or eukaryotic host (Murray, E. *et al., Nuc Acids Res.* 17:477-508, (1989)) can be selected, for example, to increase the rate of ALGF polypeptide expression or to produce recombinant RNA transcripts having desirable properties, such as a longer half-life, than transcripts produced from naturally occurring sequence.

The polynucleotide sequences of the present invention can be engineered in order to alter a ALGF coding sequence for a variety of reasons, including but not limited to, alterations which modify the cloning, processing and/or expression of the gene product. For example, alterations may be introduced using techniques which are well known in the art, e.g., site-directed mutagenesis, to insert new restriction sites, to alter glycosylation patterns, to change codon preference, to produce splice variants, etc.

The present invention also includes recombinant constructs comprising one or more of the sequences as broadly described above. The constructs comprise a vector, such as a plasmid or viral vector, into which a sequence of the invention has been inserted, in a forward or reverse orientation. In a preferred aspect of this embodiment, the construct further comprises regulatory sequences.

including, for example, a promoter, operably linked to the sequence. Large numbers of suitable vectors and promoters are known to those of skill in the art, and are commercially available. Appropriate cloning and expression vectors for use with prokaryotic and eukaryotic hosts are also described in Sambrook, *et al.*, (*supra*).

The present invention also relates to host cells which are genetically engineered with vectors of the invention, and the production of proteins and polypeptides of the invention by recombinant techniques. Host cells are genetically engineered (i.e., transduced, transformed or transfected) with the vectors of this invention which may be, for example, a cloning vector or an expression vector. The vector may be, for example, in the form of a plasmid, a viral particle, a phage, etc. The engineered host cells can be cultured in conventional nutrient media modified as appropriate for activating promoters, selecting transformants or amplifying the ALGF gene. The culture conditions, such as temperature, pH and the like, are those previously used with the host cell selected for expression, and will be apparent to those skilled in the art.

The polynucleotides of the present invention may be included in any one of a variety of expression vectors for expressing a polypeptide. Such vectors include chromosomal, nonchromosomal and synthetic DNA sequences, e.g., derivatives of SV40; bacterial plasmids; phage DNA; baculovirus; yeast plasmids; vectors derived from combinations of plasmids and phage DNA, viral DNA such as vaccinia, adenovirus, fowl pox virus, and pseudorabies. However, any other vector may be used as long as it is replicable and viable in the host. The appropriate DNA sequence may be inserted into the vector by a variety of procedures. In general, the DNA sequence is inserted into an appropriate restriction endonuclease site(s) by procedures known in the art. Such procedures and related sub-cloning procedures are deemed to be within the scope of those skilled in the art.

The DNA sequence in the expression vector is operatively linked to an appropriate transcription control sequence (promoter) to direct mRNA synthesis.

Examples of such promoters include: LTR or SV40 promoter, the *E. coli lac* or *trp* promoter, the phage lambda PL promoter, and other promoters known to control expression of genes in prokaryotic or eukaryotic cells or their viruses. The expression vector also contains a ribosome binding site for translation initiation, and a transcription terminator. The vector may also include appropriate sequences for amplifying expression. In addition, the expression vectors preferably contain one or more selectable marker genes to provide a phenotypic trait for selection of transformed host cells such as dihydrofolate reductase or neomycin resistance for eukaryotic cell culture, or such as tetracycline or ampicillin resistance in *E. coli*.

The vector containing the appropriate DNA sequence as described above, as well as an appropriate promoter or control sequence, may be employed to transform an appropriate host to permit the host to express the protein. Examples of appropriate expression hosts include: bacterial cells, such as *E. coli*, *Streptomyces*, and *Salmonella typhimurium*; fungal cells, such as yeast; insect cells such as *Drosophila* and *Spodoptera* Sf9; mammalian cells such as CHO, COS, BHK, HEK 293 or Bowes melanoma; adenoviruses; plant cells, etc. The selection of an appropriate host is deemed to be within the scope of those skilled in the art from the teachings herein. The invention is not limited by the host cells employed.

In bacterial systems, a number of expression vectors may be selected depending upon the use intended for ALGF. For example, when large quantities of ALGF are needed for the induction of antibodies, vectors which direct high level expression of fusion proteins that are readily purified may be desirable. Such vectors include, but are not limited to, multifunctional *E. coli* cloning and expression vectors such as Bluescript(R) (Stratagene), in which the ALGF coding sequence may be ligated into the vector in-frame with sequences for the amino-terminal Met and the subsequent 7 residues of beta-galactosidase so that a hybrid protein is produced; pIN vectors (Van Heeke & Schuster *J Biol Chem* **264**:5503-5509 (1989)); pET vectors (Novagen, Madison WI); and the like.

In the yeast *Saccharomyces cerevisiae* a number of vectors containing constitutive or inducible promoters such as alpha factor, alcohol oxidase and PGH may be used. For reviews, see Ausubel *et al.* (*supra*) and Grant *et al.*, *Methods in Enzymology* 153:516-544, (1987)).

In cases where plant expression vectors are used, the expression of a sequence encoding ALGF polypeptide may be driven by any of a number of promoters. For example, viral promoters such as the 35S and 19S promoters of CaMV (Brisson *et al.*, *Nature* 310:511-514, (1984)) may be used alone or in combination with the omega leader sequence from TMV (Takamatsu *et al.*, *EMBO J* 6:307-311, (1987)). Alternatively, plant promoters such as the small subunit of RUBISCO (Coruzzi *et al.*, *EMBO J* 3:1671-1680, (1984); Broglie *et al.*, *Science* 224:838-843, (1984)); or heat shock promoters (Winter J and Sinibaldi RM, *Results. Probl. Cell Differ.* 17:85-105, (1991)) may be used. These constructs can be introduced into plant cells by direct DNA transformation or pathogen-mediated transfection. For reviews of such techniques, see Hobbs S or Murry LE (1992) in McGraw Hill Yearbook of Science and Technology, McGraw Hill, New York, N.Y., pp 191-196; or Weissbach and Weissbach (1988) *Methods for Plant Molecular Biology*, Academic Press, New York, N.Y., pp 421-463.

ALGF may also be expressed in an insect system. In one such system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes in *Spodoptera frugiperda* Sf9 cells or in *Trichoplusia* larvae. The ALGF coding sequence is cloned into a nonessential region of the virus, such as the polyhedrin gene, and placed under control of the polyhedrin promoter. Successful insertion of ALGF coding sequence will render the polyhedrin gene inactive and produce recombinant virus lacking coat protein coat. The recombinant viruses are then used to infect *S. frugiperda cells* or *Trichoplusia* larvae in which ALGF is expressed (Smith *et al.*, *J Virol.*, 46:584, (1983); Engelhard EK *et al.*, *Proc Nat Acad Sci* 91:3224-3227, (1994)).

In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, a ALGF coding sequence may be ligated into an adenovirus transcription/translation complex consisting of the late promoter and tripartite leader sequence. Insertion in a nonessential E1 or E3 region of the viral genome will result in a viable virus capable of expressing ALGF in infected host cells (Logan and Shenk, *Proc Natl Acad Sci* **81**:3655-3659, (1984)). In addition, transcription enhancers, such as the rous sarcoma virus (RSV) enhancer, may be used to increase expression in mammalian host cells.

Specific initiation signals may also be required for efficient translation of a ALGF coding sequence. These signals include the ATG initiation codon and adjacent sequences. In cases where ALGF coding sequence, its initiation codon and upstream sequences are inserted into the appropriate expression vector, no additional translational control signals may be needed. However, in cases where only coding sequence, or a portion thereof, is inserted, exogenous transcriptional control signals including the ATG initiation codon must be provided. Furthermore, the initiation codon must be in the correct reading frame to ensure transcription of the entire insert. Exogenous transcriptional elements and initiation codons can be of various origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of enhancers appropriate to the cell system in use (Scharf D *et al.*, *Results Probl Cell Differ* **20**:125-62, (1994); Bittner *et al., Methods in Enzymol* **153**:516-544, (1987)).

In a further embodiment, the present invention relates to host cells containing the above-described constructs. The host cell can be a higher eukaryotic cell, such as a mammalian cell, or a lower eukaryotic cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-Dextran mediated transfection, or electroporation (Davis, L., Dibner, M., and Battey, I. (1986) Basic Methods in Molecular Biology). Cell-free translation systems can also be employed to produce

polypeptides using RNAs derived from the DNA constructs of the present invention.

A host cell strain may be chosen for its ability to modulate the expression of the inserted sequences or to process the expressed protein in the desired fashion. Such modifications of the protein include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation and acylation. Post-translational processing which cleaves a *"prepro"* form of the protein may also be important for correct insertion, folding and/or function. Different host cells such as CHO, HeLa, BHK, MDCK, 293, WI38, etc. have specific cellular machinery and characteristic mechanisms for such post-translational activities and may be chosen to ensure the correct modification and processing of the introduced, foreign protein.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express ALGF may be transformed using expression vectors which contain viral origins of replication or endogenous expression elements and a selectable marker gene. Following the introduction of the vector, cells may be allowed to grow for 1-2 days in an enriched media before they are switched to selective media. The purpose of the selectable marker is to confer resistance to selection, and its presence allows growth and recovery of cells which successfully express the introduced sequences. Resistant clumps of stably transformed cells can be proliferated using tissue culture techniques appropriate to the cell type.

Any number of selection systems may be used to recover transformed cell lines. These include, but are not limited to, the herpes simplex virus thymidine kinase (Wigler M *et al.*, *Cell* 11:223-32, (1997)) and adenine phosphoribosyltransferase (Lowy I *et al.*, *Cell* 22:817-23, (1980)) genes which can be employed in tk- or aprt- cells, respectively. Also, antimetabolite, antibiotic or herbicide resistance can be used as the basis for selection; for example, dhfr which confers resistance to methotrexate (Wigler M *et al.*, *Proc Natl Acad Sci* 77:3567-70, (1980)); npt, which confers resistance to the

aminoglycosides neomycin and G-418 (Colbere-Garapin F *et al., J Mol Biol*, **150**:1-14, (1981)) and als or pat, which confer resistance to chlorsulfuron and phosphinotricin acetyltransferase, respectively (Murry, *supra*). Additional selectable genes have been described, for example, trpB, which allows cells to utilize indole in place of tryptophan, or hisD, which allows cells to utilize histinol in place of histidine (Hartman SC and RC Mulligan, *Proc Natl Acad Sci* **85**:8047-51, (1988)). The use of visible markers has gained popularity with such markers as anthocyanins, beta-glucuronidase and its substrate, GUS, and luciferase and its substrates, luciferin and ATP, being widely used not only to identify transformants, but also to quantify the amount of transient or stable protein expression attributable to a specific vector system (Rhodes CA *et. al.*, *Methods Mol Biol* 55:121-131, (1995)).

Host cells transformed with a nucleotide sequence encoding ALGF may be cultured under conditions suitable for the expression and recovery of the encoded protein from cell culture. The protein produced by a recombinant cell may be secreted, membrane-bound, or contained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides encoding ALGF can be designed with signal sequences which direct secretion of ALGF polypeptide through a prokaryotic or eukaryotic cell membrane.

ALGF may also be expressed as a recombinant protein with one or more additional polypeptide domains added to facilitate protein purification. Such purification facilitating domains include, but are not limited to, metal chelating peptides such as histidine-tryptophan modules that allow purification on immobilized metals, protein A domains that allow purification on immobilized immunoglobulin, and the domain utilized in the FLAGS extension/affinity purification system (Immunex Corp, Seattle, WA). The inclusion of a protease-cleavable polypeptide linker sequence between the purification domain and ALGF is useful to facilitate purification. One such expression vector provides for expression of a fusion protein compromising ALGF (*e.g.*, a soluble

ALGF fragment) fused to a polyhistidine region separated by an enterokinase cleavage site. The histidine residues facilitate purification on IMIAC (immobilized metal ion affinity chromatography, as described in Porath *et al.* (1992) Protein Expression and Purification 3:263-281) while the enterokinase cleavage site provides a means for isolating ALGF from the fusion protein. pGEX vectors (Promega, Madison, WI) may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to ligand-agarose beads (e.g., glutathione-agarose in the case of GST-fusions) followed by elution in the presence of free ligand.

Following transformation of a suitable host strain and growth of the host strain to an appropriate cell density, the selected promoter is induced by appropriate means (*e.g.*, temperature shift or chemical induction) and cells are cultured for an additional period. Cells are typically harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract retained for further purification. Microbial cells employed in expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents, or other methods, which are well know to those skilled in the art.

ALGF can be recovered and purified from recombinant cell cultures by any of a number of methods well known in the art, including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography, and lectin chromatography. Protein refolding steps can be used, as necessary, in completing configuration of the mature protein. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps.

D2. Diagnostic applications

The polynucleotides of the present invention may be used for a variety of diagnostic purposes. The polynucleotides may be used to detect and quantitate

expression of ALGF or its splice variants in patient's cells, e.g. biopsied tissues, by detecting the presence of mRNA coding for ALGF receptor or its splice variants. This assay typically involves obtaining total mRNA from the tissue and contacting the mRNA with a nucleic acid probe. The probe is a nucleic acid molecule of at least 15 nucleotides, preferably at least 20 or 30 nucleotides, capable of specifically hybridizing with a sequence included within the sequence of a nucleic acid molecule encoding ALGF under hybridizing conditions, detecting the presence of mRNA hybridized to the probe, and thereby detecting the expression of ALGF. This assay can be used to distinguish between absence, presence, and excess expression of ALGF and to monitor levels of ALGF expression during therapeutic intervention.

The invention also contemplates the use of the polynucleotides as a diagnostic for diseases resulting from inherited defective ALGF genes. These genes can be detected by comparing the sequences of the defective (i.e., mutant) ALGF gene with that of a normal one. Association of a mutant ALGF gene with abnormal ALGF activity may be verified. In addition, mutant ALGF genes can be inserted into a suitable vector for expression in a functional assay system as yet another means to verify or identify mutations. Once mutant genes have been identified, one can then screen populations of interest for carriers of the mutant gene.

Individuals carrying mutations in the gene of the present invention may be detected at the DNA level by a variety of techniques. Nucleic acids used for diagnosis may be obtained from a patient's cells, including but not limited to such as from blood, urine, saliva, placenta, tissue biopsy and autopsy material. Genomic DNA may be used directly for detection or may be amplified enzymatically by using PCR (Saiki, *et al.*, *Nature* 324:163-166, (1986)) prior to analysis. RNA or cDNA may also be used for the same purpose. As an example, PCR primers complementary to the nucleic acid of the present invention can be used to identify and analyze mutations in the gene of the present invention.

Deletions and insertions can be detected by a change in size of the amplified product in comparison to the normal genotype.

Point mutations can be identified by hybridizing amplified DNA to radiolabeled RNA of the invention or alternatively, radiolabeled antisense DNA sequences of the invention. Sequence changes at specific locations may also be revealed by nuclease protection assays, such RNase and S1 protection or the chemical cleavage method (e.g. Cotton, *et al., Proc. Natl. Acad. Sci.* USA 85:4397-4401, (1985)), or by differences in melting temperatures. *"Molecular beacons"* (Kostrikis L.G. *et al., Science* 279:1228-1229, (1998)), hairpin-shaped, single-stranded synthetic oligonucleotides containing probe sequences which are complementary to the nucleic acid of the present invention, may also be used to detect point mutations or other sequence changes as well as monitor expression levels of ALGF. Such diagnostics would be particularly useful for prenatal testing.

Another method for detecting mutations uses two DNA probes which are designed to hybridize to adjacent regions of a target, with abutting bases, where the region of known or suspected mutation(s) is at or near the abutting bases. The two probes may be joined at the abutting bases, e.g., in the presence of a ligase enzyme, but only if both probes are correctly base paired in the region of probe junction. The presence or absence of mutations is then detectable by the presence or absence of ligated probe.

Also suitable for detecting mutations in the ALGF coding sequence are oligonucleotide array methods based on sequencing by hybridization (SBH), as described, for example, in U.S. Patent No. 5,547,839. In a typical method, the DNA target analyte is hybridized with an array of oligonucleotides formed on a microchip. The sequence of the target can then be *"read"* from the pattern of target binding to the array.

D3. Gene mapping

The sequences of the present invention are also valuable for chromosome identification. The sequence is specifically targeted to and can hybridize with a

particular location on an individual human chromosome. Moreover, there is a current need for identifying particular sites on the chromosome. Few chromosome marking reagents based on actual sequence data (repeat polymorphisms) are presently available for marking chromosomal location. The mapping of DNAs to chromosomes according to the present invention is an important first step in correlating those sequences with genes associated with disease.

Briefly, sequences can be mapped to chromosomes by preparing PCR primers (preferably 15 to 25 bp) from ALGF DNA. Computer analysis of the 3' untranslated region is used to rapidly select primers that do not span more than one exon in the genomic DNA, which would complicate the amplification process. These primers are then used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the primer will yield an amplified fragment.

PCR mapping of somatic cell hybrids is a rapid procedure for assigning a particular DNA to a particular chromosome. Using the present invention with the same oligonucleotide primers, sublocalization can be achieved with panels of fragments from specific chromosomes or pools of large genomic clones in an analogous manner. Other mapping strategies that can similarly be used to map to its chromosome include in situ hybridization, prescreening with labeled flow-sorted chromosomes and preselection by hybridization to construct chromosome specific-cDNA libraries.

Fluorescence *in situ* hybridization (FISH) of a cDNA clone to a metaphase chromosomal spread can be used to provide a precise chromosomal location in one step. This technique can be used with cDNA as short as 50 or 60 bases. For a review of this technique, see Verma *et al.* (1988) Human Chromosomes: a Manual of Basic Techniques, Pergamon Press, New York.

Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found, for example, in the OMIM database

(Center for Medical Genetics, Johns Hopkins University, Baltimore, MD and National Center for Biotechnology Information, National Library of Medicine, Bethesda, MD). The OMIM gene map presents the cytogenetic map location of disease genes and other expressed genes. The OMIM database provides information on diseases associated with the chromosomal location. Such associations include the results of linkage analysis mapped to this interval, and the correlation of translocations and other chromosomal aberrations in this area with the advent of polygenic diseases, such as cancer.

D4. Therapeutic applications

Polynucleotides which encode ALGF, or complements of the polynucleotides, may also be used for therapeutic purposes. Expression of ALGF may be modulated through antisense technology, which controls gene expression through complementary polynucleotides, i.e. antisense DNA or RNA, to the control, 5' or regulatory regions of the gene encoding ALGF. For example, the 5' coding portion of the polynucleotide sequence which codes for the protein of the present invention is used to design an antisense oligonucleotide of from about 10 to 40 base pairs in length. Oligonucleotides derived from the transcription start site, e.g. between positions -10 and +10 from the start site, are preferred. An antisense DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription (Lee *et al.*, *Nucl. Acids Res.* **6**:3073, (1979); Cooney *et al. Science* **241**:456 (1988); and Dervan *et al. Science* **251**: 1360, (1991)), thereby preventing transcription and the production of ALGF. An antisense RNA oligonucleotide hybridizes to the mRNA in vivo and blocks translation of the mRNA molecule into ALGF protein (Okano, *J. Neurochem.* **56**:560, (1991)). The antisense constructs can be delivered to cells by procedures known in the art such that the antisense RNA or DNA may be expressed *in vivo*.

The therapeutic polynucleotides of the invention may be employed in combination with a suitable pharmaceutical carrier. Such compositions comprise a therapeutically effective amount of the compound, and a pharmaceutically

acceptable carrier or excipient. Such a carrier includes but is not limited to saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. The formulation should suit the mode of administration.

The polypeptides, and agonist and antagonist compounds which are polypeptides, may also be employed in accordance with the present invention by expression of such polypeptides *in vivo*, which is often referred to *as "gene therapy."* Cells from a patient may be engineered with a polynucleotide (DNA or RNA) encoding a polypeptide *ex vivo*, with the engineered cells then being provided to a patient to be treated with the polypeptide. Such methods are well-known in the art. For example, cells may be engineered by procedures known in the art by use of a retroviral particle containing RNA encoding a polypeptide of the present invention.

Similarly, cells may be engineered *in vivo* for expression of a polypeptide *in vivo* by procedures known in the art. As known in the art, a producer cell for producing a retroviral particle containing RNA encoding the polypeptide of the present invention may be administered to a patient for engineering cells *in vivo* and expression of the polypeptide *in vivo*. These and other methods for administering a polypeptide of the present invention by such method should be apparent to those skilled in the art from the teachings of the present invention. For example, the expression vehicle for engineering cells may be other than a retrovirus, for example, an adenovirus which may be used to engineer cells *in vivo* after combination with a suitable delivery vehicle.

Retroviruses from which the retroviral plasmid vectors mentioned above may be derived include, but are not limited to, Moloney Murine Leukemia Virus, spleen necrosis virus, retroviruses such as Rous Sarcoma Virus, Harvey Sarcoma Virus, avian leukosis virus, gibbon ape leukemia virus, human immunodeficiency virus, adenovirus, Myeloproliferative Sarcoma Virus, and mammary tumor virus.

The retroviral plasmid vector is employed to transduce packaging cell lines to form producer cell lines. Examples of packaging cells which may be transfected include, but are not limited to, the PE501, PA317, psi-2, psi-AM,

PA12, T19-14X, VT-19-17-H2, psi-CRE, psi-CRIP, GP+E-86, GP+envAm12, and DAN cell lines as described in Miller (1990; *Human Gene Therapy*, Vol. 1, pg. 5-14). The vector may transduce the packaging cells through any means known in the art. Such means include, but are not limited to, electroporation, the use of liposomes, and $CaPO_4$ precipitation. In one alternative, the retroviral plasmid vector may be encapsulated into a liposome, or coupled to a lipid, and then administered to a host. The producer cell line generates infectious retroviral vector particles which include the nucleic acid sequence(s) encoding the polypeptides. Such retroviral vector particles then may be employed, to transduce eukaryotic cells, either in vitro or in vivo. The transduced eukaryotic cells will express the nucleic acid sequence(s) encoding the polypeptide. Eukaryotic cells which may be transduced include, but are not limited to, embryonic stem cells, embryonic carcinoma cells, as well as hematopoietic stem cells, hepatocytes, fibroblasts, myoblasts, keratinocytes, endothelial cells, and bronchial epithelial cells.

The genes introduced into cells may be placed under the control of inducible promoters, such as the radiation-inducible Egr-1 promoter, (Maceri, H.J., *et al. Cancer Res* 56(19):4311, (1996)), to stimulate ALGF production or antisense inhibition in response to radiation, *e.g.*, radiation therapy for treating tumors.

III. ALGF polypeptide

The substantially purified ALGF of the present invention includes a protein containing an amino acid sequence having at least 80%, preferably at least 90% or 95% identity to any one of the sequence identified as SEQ ID NOS:5 to 8. The protein may be a recombinant protein, a natural protein or a synthetic protein, preferably a recombinant protein. The protein may be in mature and/or modified form, also as defined above.

Also contemplated are protein fragments having at least 20 contiguous amino acid residues, preferably at least 50 residues, derived from ALGF. In one

embodiment, the fragment corresponds to the C-terminal fibrinogen-like domain of ALGF.

The sequence variations are preferably those that are considered conserved substitutions, as defined above. Thus, for example, a protein with a sequence having at least 80% sequence identity with the polypeptide identified in any one of SEQ ID NOS:5 to 8. In a more specific embodiment, the protein contains a polypeptide having a sequence substantially identical to the sequences depicted in any one of SEQ ID NOS:5 to 8. ALGF may be (i) a protein in which one or more of the amino acid residues in a sequence listed above are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue), or (ii) a protein in which one or more of the amino acid residues includes a substituent group, or (iii) a protein in which the ALGF is fused with another compound, such as a compound to increase the half-life of the protein (for example, polyethylene glycol (PEG)), or (iv) a protein in which additional amino acids are fused to ALGF, or (v) an isolated fragment of the protein which still binds its natural receptor. Such fragments, variants and derivatives are deemed to be within the scope of those skilled in the art from the teachings herein.

A. Preparation of ALGF

Recombinant methods for producing and isolating ALGF, splice variants, and fragments are described above.

In addition to recombinant production, fragments and portions of ALGF may be produced by direct peptide synthesis using solid-phase techniques (cf Stewart *et al.* (1969) Solid-Phase Peptide Synthesis, WH Freeman Co, San Francisco; Merrifield J, *J Am Chem Soc* **85**:2149-2154, (1963)). In vitro peptide synthesis may be performed using manual techniques or by automation. Automated synthesis may be achieved, for example, using Applied Biosystems 431A Peptide Synthesizer (Perkin Elmer, Foster City, Calif.) in accordance with the instructions provided by the manufacturer. Portions of ALGF may be chemically synthesized separately and combined using chemical methods.

The protein may also be obtained by isolation from natural sources, e.g., by affinity purification using the anti-ALGF antibody described in the section below. Furthermore, fragments corresponding to the N-terminal domain and/or the C-terminal domain of ALGF may be isolated using limited proteolysis techniques known to those of skill in the art. The amino acid sequence of the fragment(s) so obtained may be used to design nucleotide coding sequence for recombinant production of the fragment(s).

B. Applications of ALGF polypeptides

The ALGF growth factor of the invention has uses in (1) therapeutic treatment methods and (2) drug screening.

B1. Therapeutic uses and compositions

The ALGF protein of the present invention as well as fragments thereof are generally useful in treating diseases and disorders associated with the actions of structurally related growth factors such as ANG-1 and ANG-2.

ALGF may be employed for positive regulation of ALGF growth factor-receptor mediated processes and may be used to induce wound healing and associated therapies concerned with re-growth of tissues, such as connective tissue, neuronal tissue, hematopoietic tissue, skin, bone, cartilage, muscle, lung or kidney. ALGF may also be employed to enhance the growth of vascular smooth muscle and endothelial cells leading to the stimulation of angiogenesis. ALGF-mediated increase in angiogenesis would be beneficial to ischemic tissues and to collateral coronary development in the heart subsequent to coronary stenosis, and to other diseases characterized by vascular insufficiency. ALGF would also be beneficial in treating neuronal damage associated with, e.g., ischemia, hypoxia, or neurodegeneration resulting from stroke or cardiac arrest. ALGF may also be employed during implant fixation to stimulate the growth of cells around the implant and therefore, facilitate its attachment to its intended site.

ALGF may alternatively act as an antagonist of the angiopoietin growth factor-receptor and may thus be employed for negative regulation of angiopoietin

growth factor-receptor mediated processes. In this aspect ALGF, may be employed as an inhibitor of, for example, angiogenesis, neurogenesis, and other cellular proliferative processes. ALGF would be beneficial in inhibiting growth and metastasis of solid tumors by inhibiting tumor neovascularization. ALGF may also be used to inhibit cellular proliferation prevalent in, for example, atherosclerosis, diabetic retinopathy, arthritis, cancers including leukemias, diseases in gastrointestinal tract, infantile haemangiomas, certain disorders of development (such as intestinal artresia, vascular malformations and unilateral facial atropy), and several disorders of the female reproductive system (such as prolonged bleeding). ALGF may also be employed to inhibit the over-production of scar tissue seen in a keloid which forms after surgery, fibrosis after myocardial infarction, or fibrotic lesions associated with pulmonary fibrosis

ALGF compositions are tested in appropriate *in vitro* and *in vivo* animal models of disease, to confirm efficacy, tissue metabolism, and to estimate dosages, according to methods well known in the art.

ALGF compositions may be administered by any of a number of routes and methods designed to provide a consistent and predictable concentration of compound at the target organ or tissue. The polypeptide compositions may be administered alone or in combination with other agents, such as stabilizing compounds, and/or in combination with other pharmaceutical agents such as drugs or hormones.

ALGF compositions may be administered by a number of routes including, but not limited to oral, intravenous, intramuscular, transdermal, subcutaneous, topical, sublingual, or rectal means. ALGF compositions may also be administered via liposomes. Such administration routes and appropriate formulations are generally known to those of skill in the art.

For example, the polypeptide may be given topically to the skin or epithelial linings of body cavities, for infections in such regions. Examples of treatable body cavities include the vagina, the rectum and the urethra.

Conveniently, the polypeptide would be formulated into suppository form for administration to these areas.

The polypeptide can be given via intravenous or intraperitoneal injection. Similarly, the polypeptide may be injected to other localized regions of the body. The polypeptide may also be administered via nasal insufflation. Enteral administration is also possible. For such administration, the polypeptide should be formulated into an appropriate capsule or elixir for oral administration, or into a suppository for rectal administration.

The foregoing exemplary administration modes will likely require that the polypeptides be formulated into an appropriate carrier, including ointments, gels, suppositories. Appropriate formulations are well known to persons skilled in the art.

Dosage of the polypeptide will vary, depending upon the potency and therapeutic index of the particular polypeptide selected. These parameters are easily determinable by the skilled practitioner. As an example, if the polypeptide inhibits neuronal cell degradation *in vitro* at a given concentration, the practitioner will know that the final desired therapeutic concentration will be this range, calculated on the basis of expected biodistribution. An appropriate target concentration is in the ng/kg to low mg/kg range, e.g., 50 ng/kg to 1 mg/kg body weight, for IV administration.

A therapeutic composition for use in the treatment method can include the polypeptide in a sterile injectable solution, the polypeptide in an oral delivery vehicle, or the polypeptide in a nebulized form, all prepared according to well known methods. Such compositions comprise a therapeutically effective amount of the compound, and a pharmaceutically acceptable carrier or excipient. Such a carrier includes but is not limited to saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof.

B2. Screening methods

The present invention also includes a method for identifying a receptor for ALGF. The gene encoding the ALGF-receptor can be identified by numerous

methods known to those of skill in the art, for example, ligand panning and FACS sorting (Coligan, *et al.*, (1991) Current Protocols in Immun., 1(2), Chapter 5). Preferably, expression cloning is employed wherein polyadenylated RNA is prepared from a cell responsive to ALGF, and a cDNA library created from this RNA is divided into pools and used to transfect COS cells or other cells that are not responsive to ALGF. Transfected cells which are grown on glass slides are exposed to labeled ALGF. ALGF can be labeled by a variety of means, including iodination, or inclusion of a recognition site for a site-specific protein kinase. Following fixation and incubation, the slides are subjected to autoradiographic analysis. Positive pools are identified and sub-pools are prepared and retransfected using an iterative sub-pooling and rescreening process, eventually yielding a single clone that encodes the putative receptor.

As an alternative approach for receptor identification, labeled ALGF can be photoaffinity linked with cell membrane or extract preparations that express the receptor molecule. Cross-linked material is resolved by PAGE and exposed to X-ray film. The labeled complex containing the ALGF-receptor can be excised, resolved into peptide fragments, and subjected to protein microsequencing. The amino acid sequence obtained from microsequencing would be used to design a set of degenerate oligonucleotide probes to screen a cDNA library to identify the gene encoding the putative receptor.

The present invention also includes a method for identifying molecules, such as synthetic drugs, antibodies, peptides, or other molecules, which have a modulating effect on the activity of ALGF, *e.g.* agonists or antagonists of the ALGF-receptor. Such an assay comprises the steps of providing a cell expressing the ALGF-receptor, contacting the cell with ALGF in the presence of one or more molecules to determine its modulating effect on the activity of the receptor, and selecting from the molecules a candidate molecule capable of modulating ALGF-receptor activity. Such compounds are useful in the treatment of disease conditions associated with activation or depression of ALGF-receptor activity.

ALGF, its binding, catalytic, or immunogenic fragments, or oligopeptides thereof, can be used for screening therapeutic compounds in any of a variety of drug screening techniques. The protein employed in such a test may be membrane-bound, free in solution, affixed to a solid support, borne on a cell surface, or located intracellularly. The formation of binding complexes between ALGF or ALGF-receptor and the agent being tested may be measured, *e.g.* by surface plasmon resonance (BIACORE®; Biacore AB, Uppsala Sweden). Compounds which inhibit binding between ALGF or ALGF-receptor and its agonists may also be measured.

In one embodiment, the screening system includes recombinantly expressed ALGF, and the compounds screened are tested for their ability to block or enhance the signal transduction activity of ALGF-receptor. In a functional screening assay, mammalian cell lines which are not responsive to ALGF are used to express ALGF-receptor identified as described above. In this assay, compounds are screened for their relative affinity as receptor agonists or antagonists by comparing the relative receptor occupancy to the extent of ligand-induced stimulation or inhibition of, for example, tyrosine phosphorylation, signal transduction, or cell growth.

Another technique for drug screening which may be used provides for high throughput screening of compounds having suitable binding affinity to the ALGF protein is described in detail by Geysen in PCT Application WO 84/03564, published on September 13, 1984. In summary, large numbers of different small peptide test compounds are synthesized on a solid substrate, such as plastic pins or some other surface. The peptide test compounds are reacted with ALGF, and washed. Bound ALGF is then detected by methods well known in the art. Substantially purified ALGF can also be coated directly onto plates for use in the aforementioned drug screening techniques. Alternatively, non-neutralizing antibodies can be used to capture the peptide and immobilize it on a solid support.

Antibodies to ALGF, as described in Section IV. below, may also be used in screening assays according to methods well known in the art. For example, a "sandwich" assay may be performed, in which an anti- ALGF antibody is affixed to a solid surface such as a microtiter plate and solubilized ALGF is added. Such an assay can be used to capture compounds which bind to ALGF. Alternatively, such an assay may be used to measure the ability of compounds to interfere with the binding of a ALGF agonist to ALGF.

IV. Anti-ALGF antibodies

A. Preparation of antibodies

In still another aspect of the invention, purified ALGF is used to produce anti-ALGF antibodies which have diagnostic and therapeutic uses related to the activity, distribution, and expression of ALGF.

Antibodies to ALGF may be generated by methods well known in the art. Such antibodies may include, but are not limited to, polyclonal, monoclonal, chimeric, humanized, single chain, Fab fragments and fragments produced by an Fab expression library. Antibodies, *i.e.*, those which block ligand binding, are especially preferred for therapeutic use.

ALGF for antibody induction does not require biological activity; however, the protein fragment or oligopeptide must be antigenic. Peptides used to induce specific antibodies may have an amino acid sequence consisting of at least ten amino acids, preferably at least 20 amino acids. Preferably they should mimic a portion of the amino acid sequence of the natural protein and may contain the entire amino acid sequence of a small, naturally occurring molecule. Short stretches of a ALGF polypeptide may be fused with another protein such as keyhole limpet hemocyanin and antibody produced against the chimeric molecule. Procedures well known in the art can be used for the production of antibodies to ALGF.

For the production of antibodies, various hosts including goats, rabbits, rats, mice, etc may be immunized by injection with ALGF or any portion.

fragment or oligopeptide which retains immunogenic properties. Depending on the host species, various adjuvants may be used to increase immunological response. Such adjuvants include but are not limited to Freund's, mineral gels such as aluminum hydroxide, and surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, and dinitrophenol. BCG (bacilli Calmette-Guerin) and Corynebacterium parvum are potentially useful human adjuvants.

Monoclonal antibodies to ALGF may be prepared using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include but are not limited to the hybridoma technique originally described by Koehler and Milstein (1975; Nature 256:495-497), the human B-cell hybridoma technique (Kosbor *et al., Immunol Today* 4:72, (1983); Cote *et al., Proc Natl Acad Sci* 80:2026-2030 (1983)) and the EBV-hybridoma technique (Cole, *et al., Mol. Cell Biol.* 62:109-120 (1984)).

Techniques developed for the production of *"chimeric antibodies"*, the splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity can also be used (Morrison *et al., Proc Natl Acad Sci* 81:6851-6855 (1984); Neuberger *et al. Nature* 312:604-608 (1984); Takeda *et al. Nature* 314:452-454 (1985)). Alternatively, techniques described for the production of single chain antibodies (U.S. Pat. No. 4,946,778) can be adapted to produce single-chain antibodies specific for ALGF.

Antibodies may also be produced by inducing *in vivo* production in the lymphocyte population or by screening recombinant immunoglobulin libraries or panels of highly specific binding reagents as disclosed in Orlandi *et al.*, (*Proc Natl Acad Sci* 86:3833-3837 (1989)), and Winter *et al.* (*Nature* 349:293-299 (1991)).

Antibody fragments which contain specific binding sites for ALGF may also be generated. For example, such fragments include, but are not limited to, the F(ab')2 fragments which can be produced by pepsin digestion of the antibody

molecule and the Fab fragments which can be generated by reducing the disulfide bridges of the F(ab')2 fragments. Alternatively, Fab expression libraries may be constructed to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity (Huse WD *et al.*, *Science* 256:1275-1281 (1989)).

B. Diagnostic applications

A variety of protocols for competitive binding or immunoradiometric assays using either polyclonal or monoclonal antibodies with established specificities are well known in the art. Such immunoassays typically involve the formation of complexes between ALGF and its specific antibody and the measurement of complex formation. A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two noninterfering epitopes on ALGF is preferred, but a competitive binding assay may also be employed. These assays are described in Maddox DE *et al.* (*J Exp Med* 158:1211 (1983)).

Antibodies which specifically bind ALGF are useful for the diagnosis of conditions or diseases characterized by expression of ALGF. Alternatively, such antibodies may be used in assays to monitor patients being treated with ALGF, its agonists, or its antagonists. Diagnostic assays for ALGF protein include methods utilizing the antibody and a label to detect ALGF in extracts of cells, tissues, or biological fluids such as sera. The proteins and antibodies of the present invention may be used with or without modification. Frequently, the proteins and antibodies will be labeled by joining them, either covalently or noncovalently, with a reporter molecule. A wide variety of reporter molecules are known in the art.

A variety of protocols for measuring ALGF, using either polyclonal or monoclonal antibodies specific for the respective protein are known in the art. Examples include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and fluorescent activated cell sorting (FACS). As noted above, a two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on ALGF is preferred, but a

competitive binding assay may be employed. These assays are described, among other places, in Maddox, *et al.* (*supra*). Such protocols provide a basis for diagnosing altered or abnormal levels of ALGF expression. Normal or standard values for ALGF expression are established by combining cell extracts taken from normal subjects, preferably human, with antibody to ALGF under conditions suitable for complex formation which are well known in the art. The amount of standard complex formation may be quantified by various methods, preferably by photometric methods. Then, standard values obtained from normal samples may be compared with values obtained from samples from subjects potentially affected by disease. Deviation between standard and subject values establishes the presence of disease state.

The antibody assays are useful to determine the level of ALGF present in a particular tissue, e.g., biopsied tumor tissue or neuronal tissue, as an indication of whether ALGF is being overexpressed or underexpressed in the tissue, or as an indication of how ALGF levels are responding to drug treatment.

C. Therapeutic uses of ALGF antibodies

Therapeutic value may be achieved by administering an antibody specific against ALGF. Such antibodies may be useful in inhibiting either positive or negative ALGF-receptor interactions by, for example, preventing the interaction of ALGF with the ALGF-receptor. Anti-ALGF antibodies may be useful in therapeutic applications as outlined in Section B above.

The antibody employed is preferably a humanized monoclonal antibody, or a human Mab produced by known globulin-gene library methods. The antibody is administered typically as a sterile solution by IV injection, although other parenteral routes may be suitable. Typically, the antibody is administered in an amount between about 1-15 mg/kg body weight of the subject. Treatment is continued, e.g., with dosing every 1-7 days, until a therapeutic improvement is seen.

In one exemplary method, the antibody treatment is used to inhibit tumor growth, by administering the antibody to the site of the tumor. In another, the antibody is used to treat transplantation rejection, by administering the antibody prior to the transplantation challenge. Treatment for atherosclerosis and other proliferative conditions is also contemplated, in this case, with long term injection of antibody.

Although the invention has been described with reference to specific methods and embodiments, it is appreciated that various modifications and changes may be made without departing from the invention.

CLAIMS:

1. An isolated nucleic acid sequence selected from the group consisting of:

(i) the nucleic acid sequence depicted in a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 and SEQ ID NO:4;

(ii) nucleic acid sequences having at least 70% identity with any one of the sequences of (i); and

(iii) fragments of (i) or (ii) of at least 20 b.p.

2. A nucleic acid sequence according to Claim 1(ii) wherein the nucleic acid sequences have at least 80% identity with any one of the sequences of Claim 1(i).

3. A nucleic acid sequence according to Claim 2, wherein the nucleic acid sequences have at least 90% identity.

4. An isolated nucleic acid sequence complementary to any one of the nucleic acid sequences of Claim 1.

5. An amino acid sequence selected from the group consisting of:

(i) an amino acid sequence coded by the any one of the isolated nucleic acid sequences of Claim 1;

(ii) fragments of the amino acid sequences of (i) having at least 10 amino acids;

(iii) analogues of the amino acid sequences of (i) or (ii) in which one or more amino acids has been added, deleted, replaced or chemically modified without substantially altering the biological activity of the parent amino acid sequence.

6. An amino acid sequence according to Claim 5, as depicted in a sequence selected from the group consisting of: SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7 and SEQ ID NO:8.

7. An isolated nucleic acid sequence coding for any one of the amino acid sequences of Claim 5 or 6.

8. A purified antibody which binds specifically to any one of the amino acid sequences of Claim 5 or 6.

9. An expression vector comprising any one of the nucleic acid sequences of Claim 1 or 7 and control elements for the expression of the nucleic acid sequences in a suitable host.

10. An expression vector comprising any one of the nucleic acid sequences of Claim 4, and control elements for the expression of the nucleic acid sequence in a suitable host.

11. A host cell transfected by any one of the expression vectors of Claim 9 or 10.

12. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and as an active ingredient an agent selected from the group consisting of:

(i) the expression vector of Claim 9; and

(ii) any one of the amino acid sequences of Claim 5 or 6.

13. A pharmaceutical composition according to Claim 12, for treatment of diseases wherein a therapeutically beneficial effect is achieved by raising the level of Angiopoietin-like growth factor (ANGF).

14. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and as an active ingredient an agent selected from the group consisting of:

(i) any one of the nucleic acid sequences of Claim 4;

(ii) the expression vector of Claim 10; and

(iii) the purified antibody of Claim 8.

15. A pharmaceutical composition according to Claim 14, for treatment of diseases wherein a therapeutically beneficial effect is achieved by lowering the level of Angiopoietin-like growth factor (ANGF).

16. A method for detecting ALGF nucleic acid sequence in a biological sample, comprising the steps of:

(a) hybridizing to nucleic acid material of said biological sample at least one of the nucleic acid sequences of Claim 1 or 4; and

(b) detecting hybridization complexes;

wherein the presence of said hybridization complexes correlates with the presence of the at least one ALGF nucleic acid sequence in the said biological sample.

17. A method according to Claim 16, wherein the nucleic acid material of said biological sample are mRNA transcripts.

18. A method according to Claim 16, wherein the nucleic acid sequences of Claim 1 or 4 are present in a nucleic acid chip.

19. A method for identifying candidate compounds capable of binding to ALGF protein and modulating its activity the method comprising:

(i) providing a protein or polypeptide comprising an amino acid sequence substantially as depicted in any one of SEQ ID NO: 5 to SEQ. ID NO: 8, or a fragment of such a sequence;

(ii) contacting a candidate compound with said amino acid sequence;

(iii) determining the effect of said candidate compound on the biological activity of the protein and selecting those compounds which show a significant effect on said biological activity.

20. A method according to Claim 19, wherein the compound is an agonist of ALGF and the measured effect is increase in signal transduction activity of ALGF protein above a selected threshold level.

21. A method according to Claim 19, wherein the compound inhibits binding of ALGF agonist to ALGF and the measured affect is decrease in the extent of binding of agonist to ALGF in the presence of the compound as compared to the extent of binding in the above of the compound.

22. An agonist of any one of the amino acid sequences of Claim 5 or 6.

23. An antagonist of any one of the amino acid sequences of Claim 5 or 6.

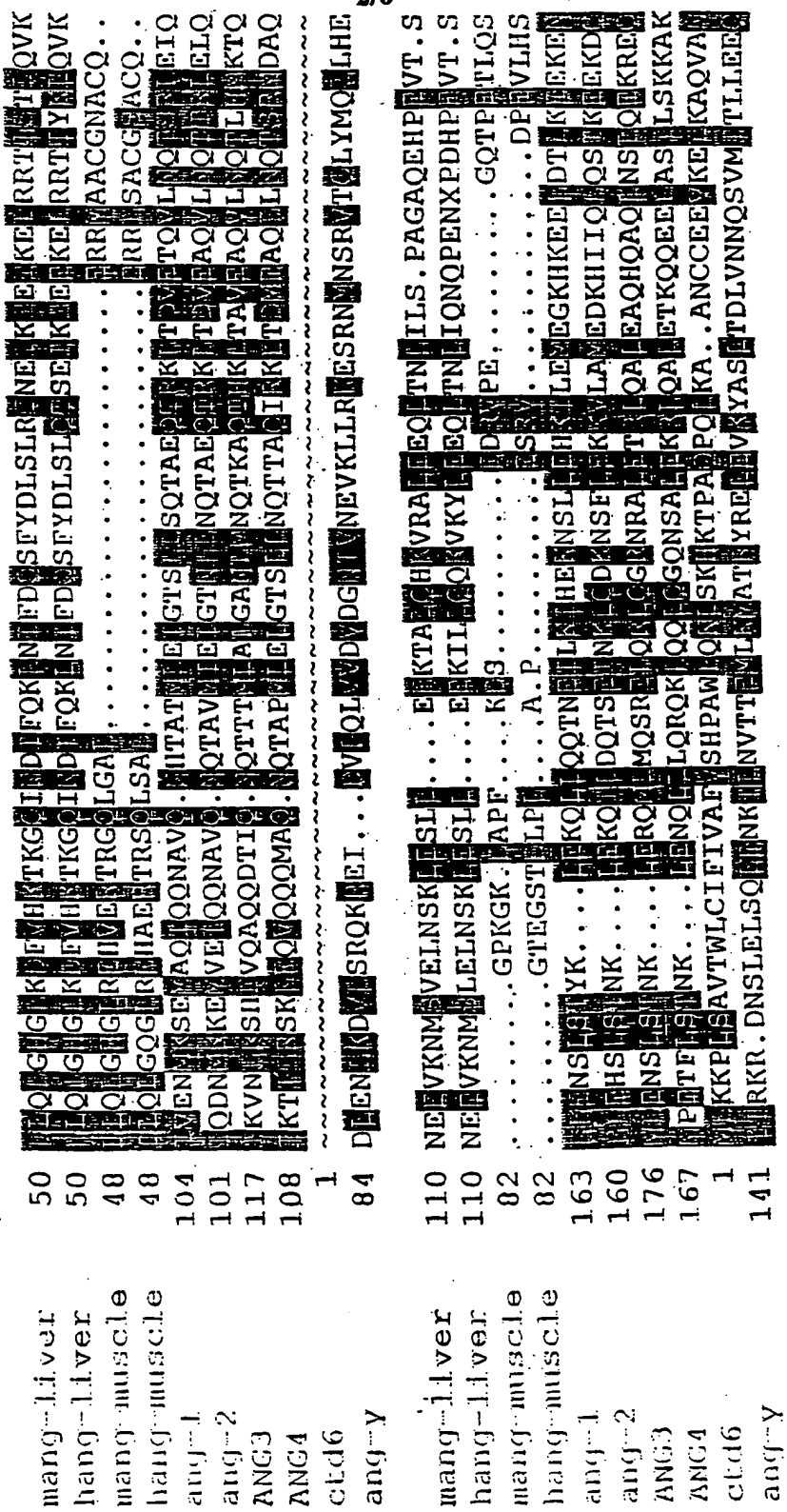
24. A method for detecting ALGF in a biological sample, comprising the steps of:

(a) contacting with said biological sample the antibody of Claim 8, thereby forming an antibody-antigen complex; and

(b) detecting said antibody-antigen complex

wherein the presence of said antibody-antigen complex correlates with the presence of ALGF in said biological sample.

Fig. 1



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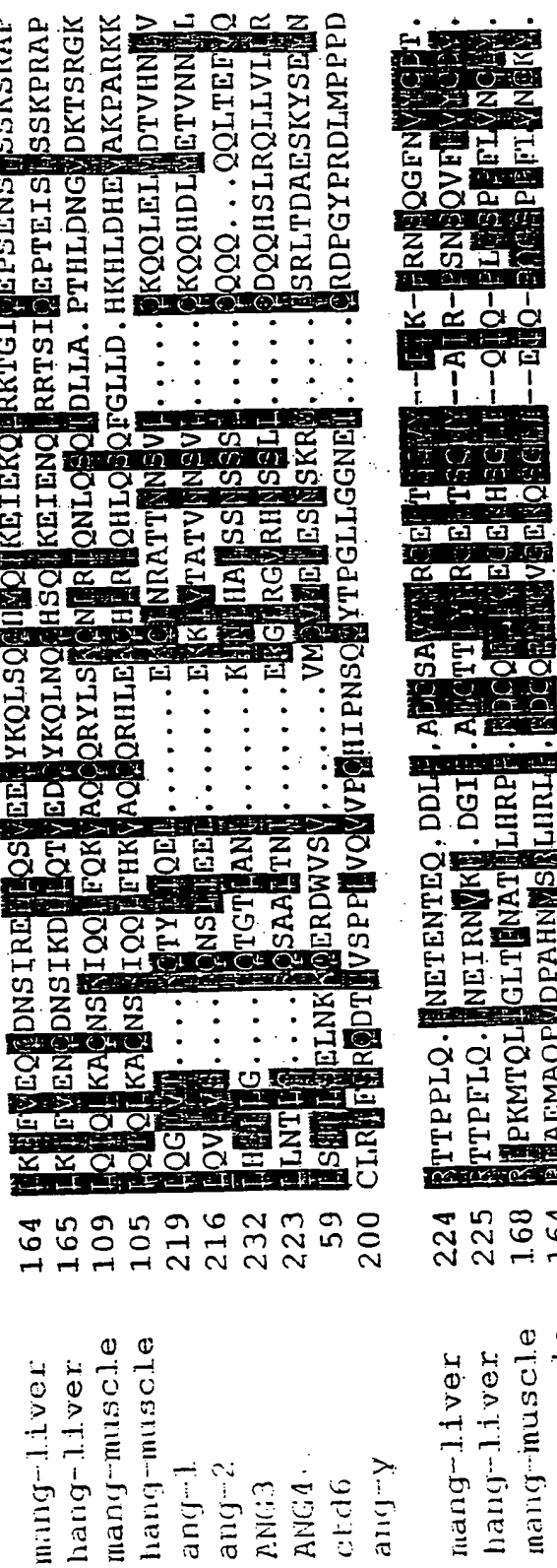

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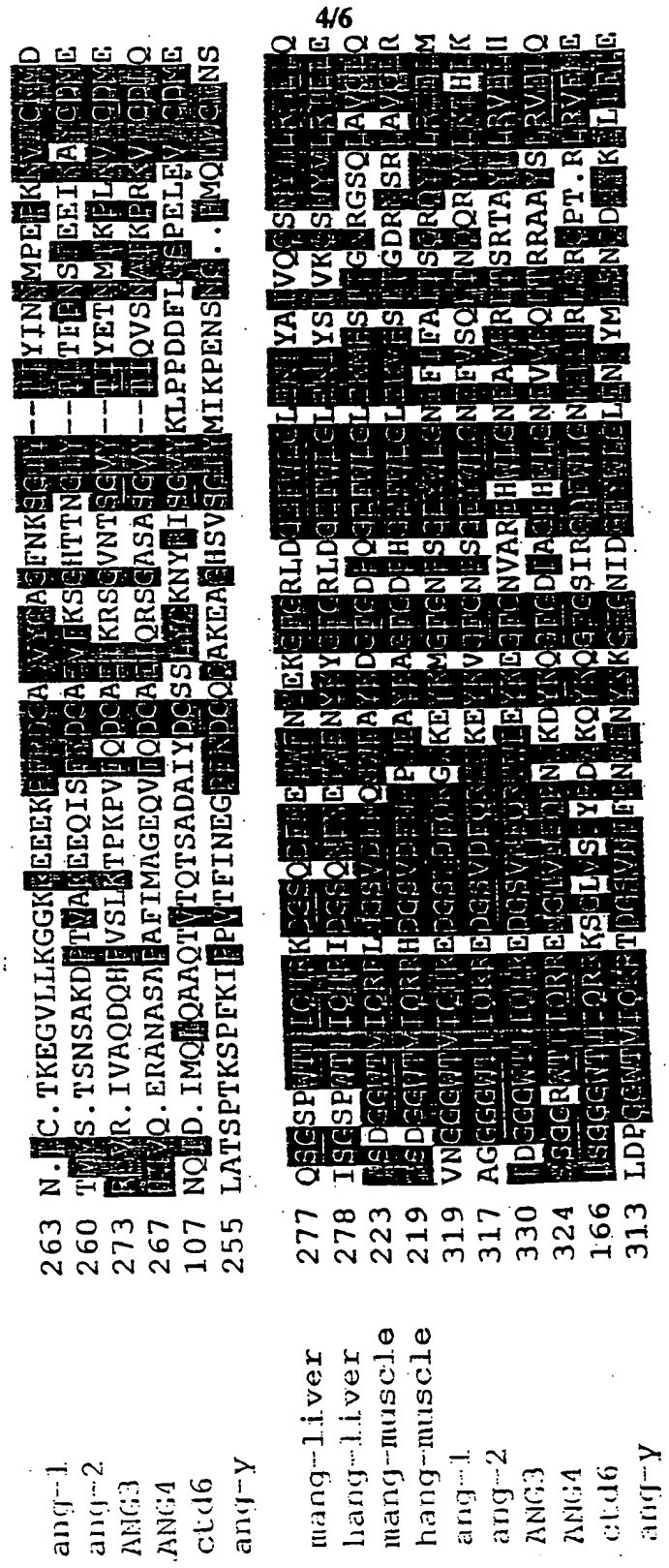

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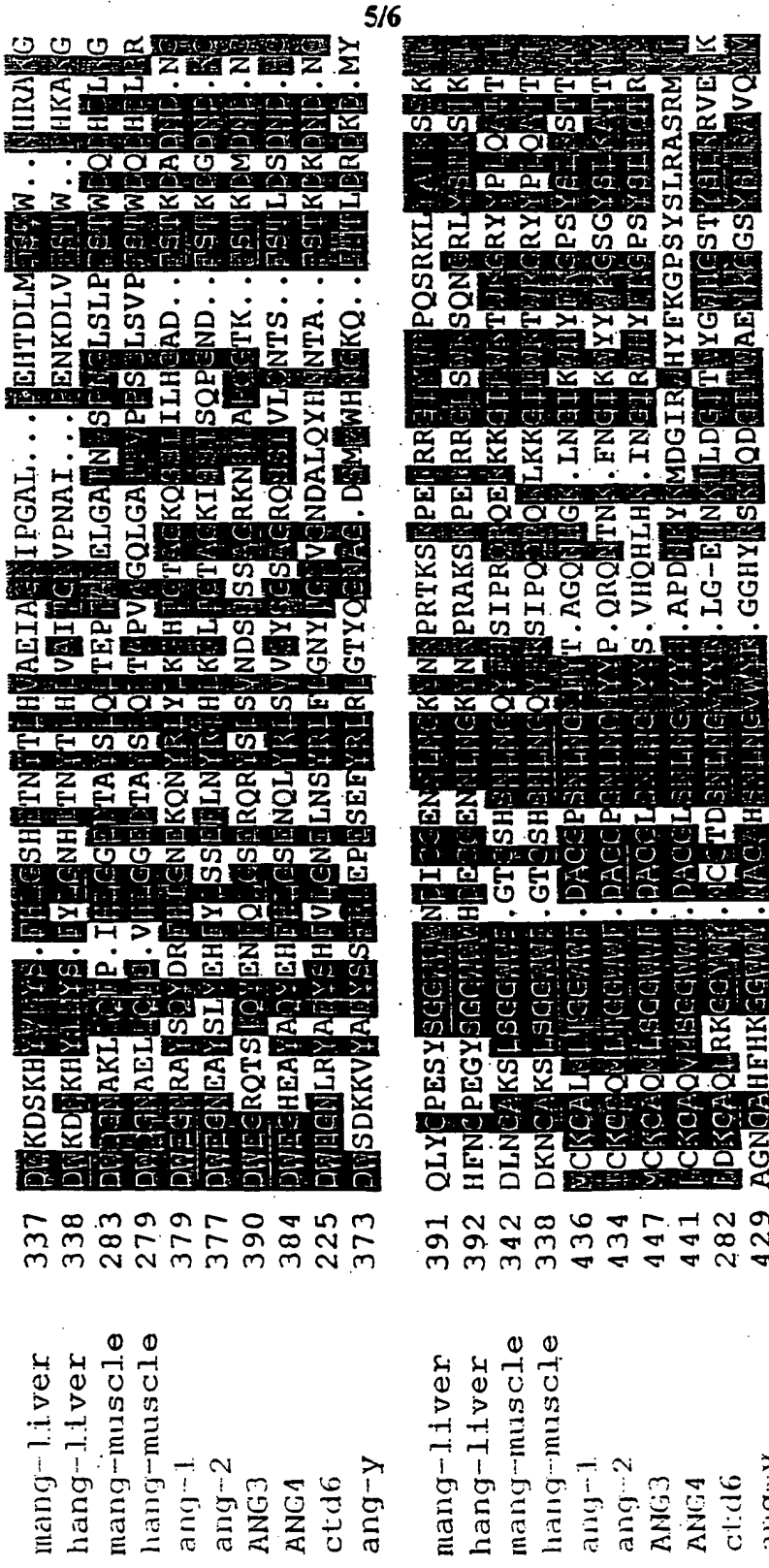
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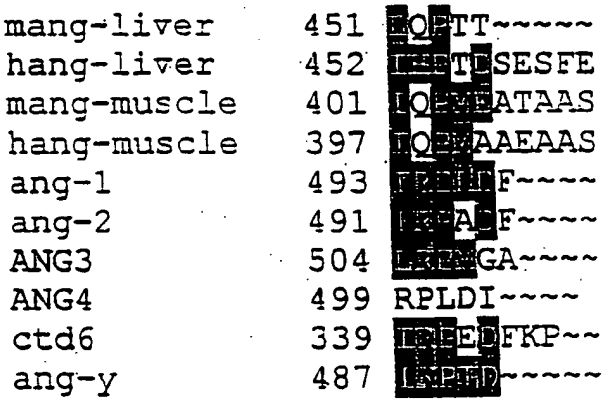
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Fig. 1 (Cont.)

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(51) **International Patent Classification** 6 :
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A3

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(71) **Applicant** *(for all designated States except US)*: COMPUGEN LTD. [IL/IL]; Pinchas Rozen Street 72, 69512 Tel-Aviv (IL).

(72) **Inventors; and**
(75) **Inventors/Applicants** *(for US only)*: MINTZ, Liat [IL/IL]; Harav Kook Street 14, 47283 Ramat Hasharon (IL). SAVITSKY, Kinneret [IL/IL]; Jacob Street 16, Tel Baruch, 69015 Tel-Aviv (IL).

(74) **Agent:** REINHOLD COHN AND PARTNERS; P.O. Box 4060, 61040 Tel-Aviv (IL).

(81) **Designated States:** AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

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(54) **Title:** ANGIOPOIETIN-LIKE GROWTH FACTOR SEQUENCES

(57) **Abstract**

The present invention concerns novel nucleic acid and amino acid sequences, expression vectors, host cells and pharmaceutical compositions comprising same, as well as antibodies directed against said amino acid sequences and methods for detecting the amino acid sequences and the nucleic acid sequences in a sample.

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CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
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EE	Estonia	LR	Liberia	SG	Singapore		

INTERNATIONAL SEARCH REPORT

Inte onal Application No
PCT/IL 99/00343

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/12 C12N5/10 C12N1/21 C07K14/515 C07K16/22
A61K31/70 A61K38/18 A61K48/00 C12Q1/68 G01N33/50
G01N33/53

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C07K A61K C12Q G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE EMEST 30 E.M.B.L. Databases Accession Number: T08223, 5 August 1993 (1993-08-05) ADAMS M ET AL: "Homo sapiens cDNA clone HIBBA76 5' end similar to Fibrinogen, beta chain" XP002118808 99.2% identity in 353nt overlap with SeqIdNo.1 abstract --- -/--	1-7

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[X] Patent family members are listed in annex.

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
1 February 2000	23. 02. 2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Lonnoy, O

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Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inte onal Application No
PCT/IL 99/00343

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE EMEST29 E.M.B.L. Databases Accession Number: AA048870, 28 November 1996 (1996-11-28) MARRA M ET AL: "Mus musculus cDNA clone 479570 5' similar to PIR: JN0596 JN0596 fibrinogen-related protein HFREP-1 precursor" XP002118809 99.8% identity in 458nt overlap with SeqIdNo.2 abstract ---	1-7
X	DATABASE EMEST4 E.M.B.L. Databases Accession Number: AA919959, 24 April 1998 (1998-04-24) MARRA M ET AL: "Mus musculus cDNA clone 1298657 5' similar to TR:Q28763 FIBRINOGEN ALPHA-E SUBUNIT" XP002129381 100% identity in 433 nt overlap with SeqIdNo.4 abstract ---	1-7
X	WO 93 00353 A (US ARMY) 7 January 1993 (1993-01-07) SeqIdNo.45: 94.8% identity in 286bp overlap with SeqIdNo.1 ---	1-4,7
P,X	WO 99 15654 A (FERRARA NAPOLEONE ;WILLIAMS P MICKEY (US); GENENTECH INC (US); FON) 1 April 1999 (1999-04-01) SeqIdNo.1: 99.9% identity in 1859 nt overlap with SeqIdNo.1 SeqIdNo.2: 99.5% identity in 406 aa overlap with SeqIdNo.5 ---	1-24
P,X	WO 99 15653 A (FERRARA NAPOLEONE ;GENENTECH INC (US); SCHWALL RALPH (US); BOTSTEI) 1 April 1999 (1999-04-01) SeqIdNo.16: 98.3% identity in 1528nt overlap with SeqIdNo.3 SeqIdNo.17: 100% identity in 460aa overlap with SeqIdNo.7 ---	1-7
E	WO 99 32515 A (ZYMOGENETICS INC) 1 July 1999 (1999-07-01) SeqIdNo.1: 99.9% identity in 1858 nt overlap with SeqIdNo.1 SeqIdNo.2: 99.8% identity in 406 aa overlap with SeqIdNo.5 --- -/--	1-24

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INTERNATIONAL SEARCH REPORT

Int. ional Application No
PCT/IL 99/00343

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category '	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	WO 97 48804 A (REGENERON PHARMA ;VALENZUELA DAVID M (US); JONES PAMELA F (GB); YA) 24 December 1997 (1997-12-24) claims 4,23 ---	
A	WO 98 05779 A (REGENERON PHARMA) 12 February 1998 (1998-02-12) claim 1 ---	
A	WO 96 31598 A (GOLDFARB MITCHELL ;BRUNO JOANNE (US); DAVIS SAMUEL (US); JONES PAM) 10 October 1996 (1996-10-10) claim 3 ---	
A	HANAHAN D: "Signaling Vascular Morphogenesis and Maintenance" SCIENCE, vol. 277, 4 July 1997 (1997-07-04), pages 48-50, XP002118807 -----	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IL 99/00343

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [ ] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. [X] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

Claims 22 and 23, respectively relating to an agonist/antagonist of the polypeptide of claim 5 or 6, could not be searched completely as the corresponding subject-matters were not sufficiently disclosed.

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. [X] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[X] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims 22 and 23, respectively relating to an agonist/antagonist of the polypeptide of claim 5 or 6, could not be searched completely as the corresponding subject-matters were not sufficiently disclosed.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-24 (all partially)

An isolated nucleic acid sequence selected from the group consisting of a nucleic acid sequence of the so-called muscle ALGF gene, said sequence having the sequence of SeqIdNo.1, said sequence having the sequence of SeqIdNo.2, sequences at least 70%, 80% or 90% identical to SeqIdNo.1 or 2, fragments of at least 20 nucleotides thereof, and sequences complementary thereto; An amino acid sequence selected from the group consisting of an amino acid sequence of the so-called muscle ALGF protein, an amino acid sequence coded by one of said nucleotide sequences, fragments of at least 10 amino acids thereof, analogues thereof, an amino acid sequence consisting of SeqIdNo.5, and an amino acid sequence consisting of SeqIdNo.6; Antibodies, expression vectors, host cells, pharmaceutical compositions, detection methods, diagnostic methods, agonists and antagonists relating thereto.

2. Claims: 1-24 (all partially)

Idem as for subject 1 but respectively relating to the so-called liver ALGF gene and protein sequences, SeqIdNo.3, SeqIdNo.4, SeqIdNo.7 and SeqIdNo.8

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte onal Application No
PCT/IL 99/00343

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9300353 A	07-01-1993	AU 2240492 A	25-01-1993
		EP 0593580 A	27-04-1994
		AU 3665893 A	03-09-1993
		WO 9316178 A	19-08-1993
WO 9915654 A	01-04-1999	AU 9388198 A	12-04-1999
WO 9915653 A	01-04-1999	US 5972338 A	26-10-1999
		AU 9388098 A	12-04-1999
WO 9932515 A	01-07-1999	NONE	
WO 9748804 A	24-12-1997	US 5851797 A	22-12-1998
		AU 3406197 A	07-01-1998
		CN 1227603 A	01-09-1999
		CZ 9804218 A	12-05-1999
		EP 0939809 A	08-09-1999
		FI 982745 A	04-02-1999
		NO 985904 A	19-02-1999
		PL 330705 A	24-05-1999
		AU 3968797 A	25-02-1998
		CN 1232501 A	20-10-1999
		EP 0915974 A	19-05-1999
		NO 990470 A	06-04-1999
		PL 331405 A	19-07-1999
		WO 9805779 A	12-02-1998
WO 9805779 A	12-02-1998	AU 3406197 A	07-01-1998
		AU 3968797 A	25-02-1998
		CN 1227603 A	01-09-1999
		CN 1232501 A	20-10-1999
		CZ 9804218 A	12-05-1999
		EP 0939809 A	08-09-1999
		EP 0915974 A	19-05-1999
		FI 982745 A	04-02-1999
		NO 985904 A	19-02-1999
		NO 990470 A	06-04-1999
		PL 330705 A	24-05-1999
		PL 331405 A	19-07-1999
		WO 9748804 A	24-12-1997
WO 9631598 A	10-10-1996	US 5814464 A	29-09-1998
		AU 711111 B	07-10-1999
		AU 4129596 A	02-05-1996
		AU 5387196 A	23-10-1996
		CA 2202028 A	18-04-1996
		CA 2216963 A	10-10-1996
		CN 1167505 A	10-12-1997
		CZ 9701025 A	17-09-1997
		EP 0784683 A	23-07-1997
		EP 0821728 A	04-02-1998
		FI 971406 A	04-06-1997
		HU 77491 A	28-05-1998
		JP 10509583 T	22-09-1998
		JP 11503323 T	26-03-1999
		NO 971557 A	06-06-1997
		NZ 296638 A	29-03-1999
		PL 319586 A	18-08-1997

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/IL 99/00343

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9631598 A		WO 9611269 A	18-04-1996